Houk and Dr. Tahsin Chow for helpful discussions.

Registry No. **2a**, 2175-91-9; **2b**, 2175-90-8; **7a**, 2860-54-0; **7b**, 141665-68-1; **8a**, 141684-30-2; **8b**, 141665-69-2; **9a**, 141684-31-3; **9b**, 141665-70-5; **9c**, 141665-72-7; **10a**, 141665-62-5; **10b**, 141665-71-6; **11**, 141665-63-6; **12**, 141665-64-7; **13**, 141665-65-8; **14**, 141665-66-9; **15**, 141665-67-0; dimethyl acetylenedicarboxylate, 762-42-5.

Kinetics of a Heterogeneous Enzymatic Hydrolysis of a Prochiral Diester

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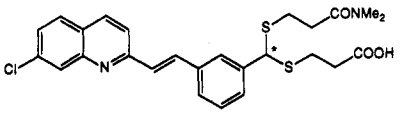
Kinetics of a Heterogeneous Enzymatic Hydrolysis of a Prochiral Diester. An enantioselective enzymatic hydrolysis of a prochiral diester was devised in our laboratories as the key step in short, efficient syntheses of both enantiomers of leukotriene D_4 antagonist MK-0571 **1**, being investigated as a therapeutic agent for bronchial disease.[1]



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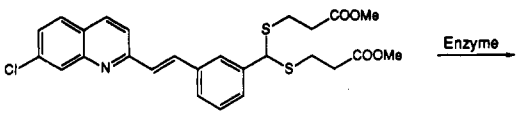

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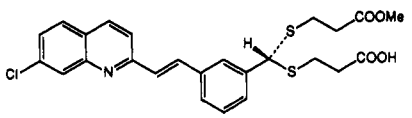

3

Lipase from *Pseudomonas* species cleanly hydrolyzed diester **2** to (*S*)-ester–acid **3** in >98% enantiomeric excess and 95% yield, with overreaction of only 2% to the corresponding diacid. The chemically and optically pure **3** was readily converted into either enantiomer of **1**. These studies have been described in detail along with further examples of successful enzymatic hydrolyses of similar esters having remote chiral/prochiral centers up to five bonds away from the ester carbonyl group.[2] We present here a quantitative analysis of the kinetics of enzymatic hydrolysis of **2** wherein enzyme inhibition by the hydrolysis product plays an important role.

In the key hydrolysis step, solid **2** is slurried in a solution of the lipase and surfactant Triton X-100 (used to speed the reaction) in 0.1 M pH 7.5 aqueous phosphate buffer at 40 °C. Analyses revealed that both **2** and **3** became supersaturated and then precipitated during the reaction, and the hydrolysis rate was quite an unusual function of the reaction time. Kinetic studies of the simpler homogeneous hydrolysis, carried out at low initial **2** concentration, allowed us to explain the kinetic data of the heterogeneous reaction mixtures.

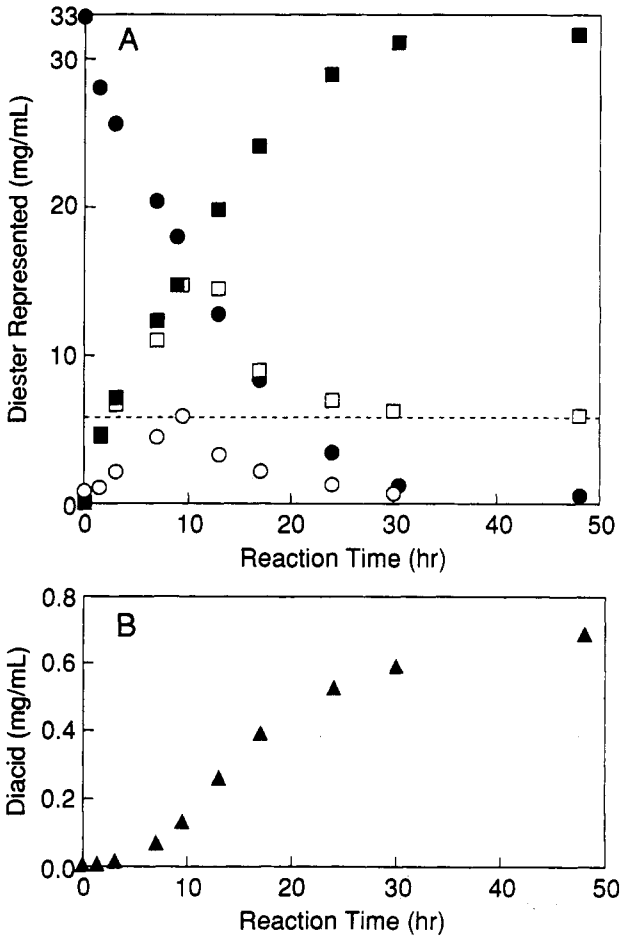

Figure 1. (A) Heterogeneous hydrolysis of diester **2** (1 g) in 0.1 M pH 7.5 phosphate buffer (30 mL) containing Triton X-100 (18 mg/mL) and the lipase (1.33 mg/mL) at 40 °C. Key: (●) total **2**; (○) **2** in filtrate; (■) total **3**; (□) **3** in filtrate; (---) solubility of **3**. (B) Expanded presentation of the diacid data.

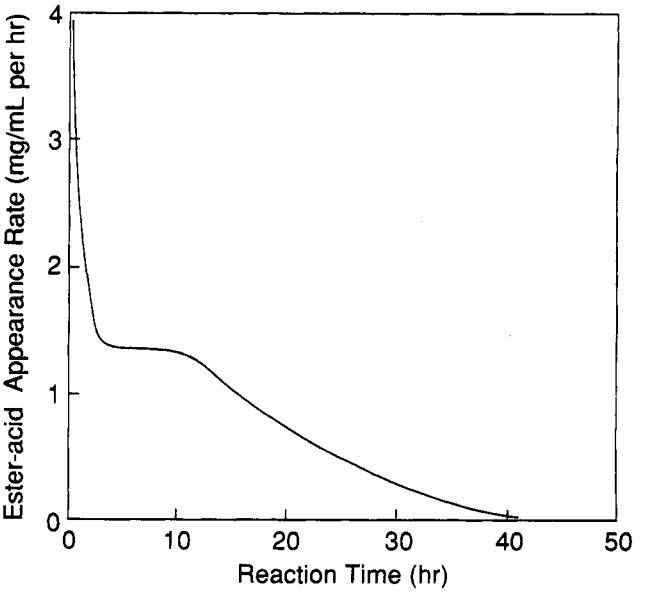

Figure 2. Appearance rate of **3** in heterogeneous hydrolysis of **2** (1 g) in 0.1 M pH 7.5 phosphate buffer (30 mL) containing Triton X-100 (18 mg/mL) and the lipase (1.33 mg/mL) at 40 °C.

Results and Discussion

Kinetic data for **2** and **3**, both the total amounts present and concentrations in filtrates, are shown in Figure 1A for

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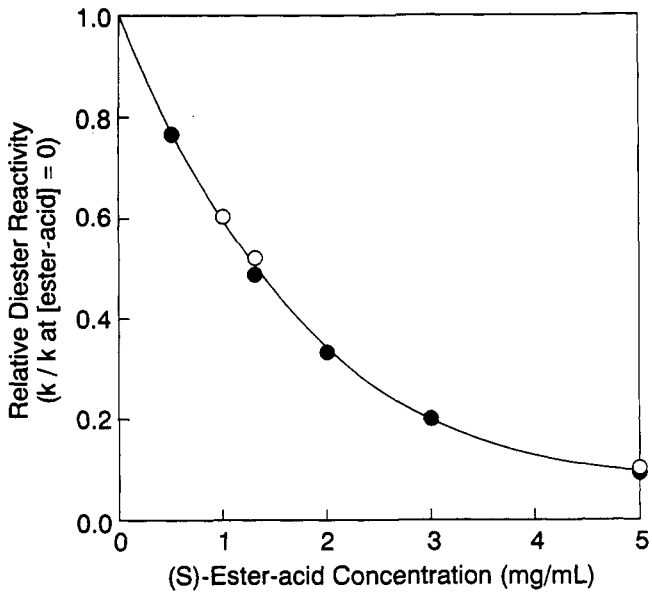


Figure 3. Enzyme inhibition by (*S*)-ester–acid **3** in homogeneous hydrolyses of **2**. Key: Lipase concentration (●) 1.33 mg/mL; (○) 0.67 mg/mL.

a 30-mL hydrolysis mixture containing 1 g of **2** initially. The appearance rate of **3** during the hydrolysis, evaluated as the slope of the plot of total **3** vs reaction time, is shown in Figure 2. (For the sake of clarity, some data points are not included in Figure 1. All of the kinetic data are available as supplementary material.) The rate declined sharply during the first 3 h and then remained nearly constant for about 9 h. In these early stages, **3** was found entirely in solution, becoming supersaturated and reaching a filtrate concentration of about 15 mg/mL after 12 h. The filtrate concentration of **2** increased from 0.8 mg/mL at the beginning of the hydrolysis to about 6 mg/mL. Then the filtrate concentrations of both **2** and **3** decreased slowly, the latter approaching the solubility value (see Experimental Section), and the hydrolysis rate diminished. The concentration of the diacid, found entirely in solution, is plotted on an expanded ordinate scale in Figure 1B. In otherwise identical hydrolyses with 0.5 or 0.25 g of **2**, **3** formed at the same rate as in the 1-g runs until **2** was largely depleted.

While some of the unusual kinetic behavior seemed to be due to the varying solution concentration of **2**, the sharply decreasing hydrolysis rate from the start must have another explanation, most reasonably, that the product **3** inhibits the reaction. Compound **3** was absent initially, of course, but soon became a major component in solution. In order to explore this, hydrolyses were carried out at low substrate concentration so that the reactant and product were completely dissolved, thus simplifying the kinetic analysis.

The rate effect of **3** was determined in dilute, homogeneous hydrolyses using the same composition of Triton X-100, the lipase, and the buffer, with **2** dissolved initially at 0.5 mg/mL together with **3** up to 5 mg/mL. Plots of log [**2**] vs reaction time were nearly linear, and the apparent pseudo-first-order reactivity of **2** at any given **3** concentration was found to be proportional to the lipase concentration. The results for kinetic runs at two lipase concentrations are summarized in a plot of the initial reactivity of **2** relative to that observed with no added **3** vs the **3** concentration, Figure 3, including a curve drawn to fit the data. (At 1.33 mg/mL of the lipase the apparent reactivity of **2** with no added **3** was 5.7 h^{-1}.) The reactivity of **2** was reduced to half of its uninhibited reactivity with

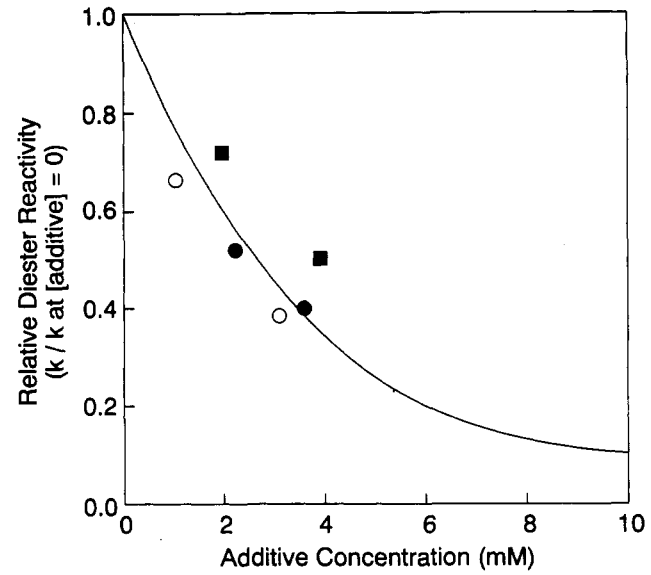
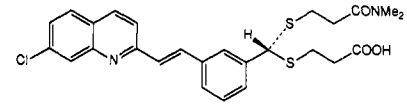


Figure 4. Enzyme inhibition by several additives in homogeneous hydrolyses of **2** at 1.33 mg lipase per mL. Key: (●) racemic ester–acid; (○) diacid; (■) **4**; (—) curve for **3**.

3 at 1 mg/mL, and to one-tenth of its uninhibited reactivity at 5 mg/mL. These concentrations of **3** are reached early in the 50-h, 1-g heterogeneous hydrolysis, at reaction times of only 0.2 and 1.5 h. Thereafter, **3** becomes even more concentrated, severely inhibiting the reaction.

The lipase and **3** were shown to be in rapid equilibrium. In homogeneous hydrolyses initiated by adding **2** last, the rate was the same when the lipase and **3** had been dissolved for an hour or just a few minutes. Moreover, when a hydrolyzing solution was diluted with an equal volume of the Triton X-100/buffer solvent mixture, a new log-linear rate became evident immediately, reflecting both the reduced lipase concentration and the weaker inhibition indicated in Figure 3 for the diluted **3**. A simple mathematical model was found to be consistent with the product inhibition data, provided that the interaction of the lipase and **3** involves more than a single equilibrium (see Appendix).

Enzyme inhibition in dilute, homogeneous hydrolyses was observed also with the racemic ester–acid, the corresponding diacid, or **4** (the enantiomer of **1**) present initially



4

instead of **3**. (The (*R*)-ester–acid and diacid were minor byproducts of the diester hydrolysis. Compound **4** was used as another carboxylic acid of similar structure and size.) Those inhibitors are compared with **3** using a millimolarity abscissa scale in Figure 4. Inhibition by the racemic ester–acid was measurably the same as that by **3**. Inhibition by the diacid was somewhat stronger and that by **4** somewhat weaker. Thus, the roles of the (*R*)-ester–acid and diacid as inhibitors in the heterogeneous hydrolysis of **2** were found relatively unimportant since their solution concentrations were much less than that of **3**.

The three distinct stages of the slurry hydrolysis evident in Figure 2 may now be rationalized. The slowing in the first stage was due to generation of the inhibiting product. In the second stage the increasing concentration of **2** offset the increasing concentration of **3**, and the hydrolysis rate remained nearly constant. In the third stage the concen-

trations of both 2 and 3 decreased such that the rate dropped slowly. In support of this rationale, the steady second-stage rate was observed from the start in a 1-g hydrolysis of 2 with the solution phase saturated with 3 initially.

Experimental Section

Materials. Acetonitrile (Fisher HPLC-grade) and trifluoroacetic acid (Fisher certified) were used to prepare the HPLC mobile phase. Triton X-100 (Roehm and Haas), lipase (Amano LPL-80 with activity of 889 000 u/g), and aqueous phosphate buffer, prepared from potassium phosphate dibasic (Fisher certified ACS) and concentrated HCl (Mallinckrodt Analytical Reagent), were used for hydrolysis reaction mixtures. Compounds 2, 3, the corresponding racemic ester–acid and diacid, and 4, all described previously,[1,2] were available in these laboratories.

Kinetics Runs. In heterogeneous enzymatic hydrolyses, solid diester 2 (1.00, 0.50, or 0.25 g) was added to 0.1 M pH 7.5 phosphate buffer (30 mL) containing Triton X-100 (0.5 mL). The slurries were stirred mechanically and thermostated in a water bath at 40 °C or were agitated using an ultrasonic probe (Heat Systems-Ultrasonics, Inc., Model W-370). The probe was used in a reaction vessel equipped with a circulating water jacket kept at 36 °C to compensate for heat generated by the probe, and the reaction temperature of about 40 °C was monitored using a thermocouple. The hydrolysis was found to proceed the same with mechanical stirring or ultrasonic agitation; mechanical stirring was preferred for better control of the reaction temperature.

In preliminary runs, assays of stirred mixtures of the buffer, Triton X-100, and 2 or 3, without enzyme, showed initial supersaturation followed by equilibration within a half hour to filtrate concentrations (solubilities) of 0.8 mg/mL of 2 or 6 mg/mL of 3. In hydrolysis kinetic runs the lipase (40 mg) was added last to initiate hydrolysis after stirring for a half hour. Aliquots of the slurry, taken periodically while stirring, were analyzed for the diester, ester–acid, and diacid using HPLC; see below. Aliquots of the solution phase, obtained by filtration through an immersed fine-porosity glass frit, were analyzed similarly.

In homogeneous enzymatic hydrolyses, solutions of Triton X-100 (0.5 mL) and diester 2 (15 mg) in the pH 7.5 buffer (30 mL) were thermostated in the water bath at 40 °C, and again the lipase (20 or 40 mg) was added last to initiate hydrolysis. Some hydrolyses of 2 were performed with 3, the racemic ester–acid, the diacid, or 4 present initially.

High-Performance Liquid Chromatography. A 250- × 4.6-mm Partisil 10 ODS column (Whatman) was used for liquid chromatography with an isocratic mobile phase of acetonitrile/0.2 wt % aqueous trifluoroacetic acid 44:56 v/v, delivered at a rate of 2 mL/min with the column at ambient temperature (23 °C). Compounds 2, 3 (or the racemate), the diacid, and 4 eluted at retention times of 9, 5, 3, and 4 min, respectively, and all exhibited the same UV spectrum. Chromatograms were monitored at 292 nm, chosen as a peak wavelength at which Triton X-100 and the lipase did not absorb.

Appendix

The reversible inhibition of enzyme E by (*S*)-ester–acid product P was modeled assuming rapid equilibria involving unreactive complexes EP_1 and EP_2 in solution.

$$E + P \rightleftharpoons EP_1 \qquad K_1 = [EP_1]/[E][P] \qquad (1)$$

$$EP_1 + P \rightleftharpoons EP_2 \qquad K_2 = [EP_2]/[EP_1][P] \qquad (2)$$

Hydrolysis of diester substrate S was assumed to occur via complex ES, in rapid equilibrium also.

$$E + S \rightleftharpoons ES \qquad K_S = [ES]/[E][S] \qquad (3)$$

The apparent diester reactivity relative to that in the absence of P (the ordinate function in Figure 3) was calculated as $[E]/[E_{total}]$. Assuming [ES] low for the sake of simplicity, EP_1 and EP_2 were eliminated by substitution from eqs 1 and 2 and the enzyme material balance, eq 4, to obtain eq 5.

$$[E_{total}] = [E] + [EP_1] + [EP_2] \qquad (4)$$

$$[E]/[E_{total}] = 1/[1 + K_1[P]^1 + K_1K_2[P]^2] \qquad (5)$$

This function of [P], evaluated for various values of the *K*'s, was compared to the relative reactivity data curve, Figure 3. No good fit was obtained assuming a single equilibrium , eq 1, but calculated curves fitting the data curve were obtained using two equilibria, eqs 1 and 2. For example, the curve for $K_1 = K_2 = 0.5$ (mg/mL)$^{-1}$ fit, but considerable variation was found tolerable. The agreement was just as good with K_1 two or three times larger and K_2 moderately smaller, or with K_1 two or three times smaller and K_2 larger. Since [P] exceeded the solubility value of 6 mg/mL during most of the heterogeneous hydrolysis, *K* values of 0.5 connote $[EP_1]/[E]$ and $[EP_2]/[EP_1]$ ratios greater than 3, eqs 1 and 2.

Acknowledgment. The authors thank Dr. D. L. Hughes for helpful discussions and Mrs. H. R. Graham for typing the manuscript.

Cross Coupling Reactions of 2-(Allyloxy(thio))benzothiazoles with Organocopper Reagents in Dihydropyranoid Systems. Mechanistic Implications of the Substrate and the Reagent: Regio- and Stereocontrolled Access to Branched-Chain Sugars

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Allylic derivatives are very useful compounds from a synthetic, mechanistic and biochemical point of view.[1] One of the chemical aspects which has attracted more attention is the regio- and stereochemistry of nucleophilic displacement reactions, and in particular those related to the applications of organometallic reagents in the selective formation of C,C bonds. In general, little regioselectivity has been achieved when working under stoichiometric conditions, and the regioselectivity of the reaction has been shown to depend on such factors as solvent, substrate, reagents, etc. Goering et al.[2a-d] have achieved a high degree of regiocontrol by using allylic carboxylates in the presence of Grignard reagents and catalytic amounts of cuprous cyanide. The stereochemistry of this reaction with regard to the relative disposition of the metal and the leaving groups is predominantly anti, as in most reactions of allylic electrophiles with electron-rich complexes.[2e] For these particular substrates these authors have postulated the formation of a σ-copper(III) complex (A) which can either undergo stereospecific reductive elimination to give anti-γ alkylation or isomerize to the π-allyl complex (B) (Scheme I). The active species are considered to be RCu(Z)MgBr

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